A genetic perspective on the specific status of the Western Rock Lobster along the coast of Western Australia – *Panulirus cygnus* George 1962 or *P. longipes* A Milne-Edwards 1868?

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Abstract

Panulirus longipes is widespread throughout the the Indo-west Pacific and *P. cygnus* is restricted to the coast of Western Australia. Controversy regarding their discreteness has continued since the early 1900's based on the lack of morphological differences and the potential for gene-flow between these species. Allozyme variation indicates a discreteness of *P. cygnus* and *P. longipes* along the coast of Western Australia. Unique alleles were found at the *PGM* locus in *P. longipes*. At 3 other loci (*EST*, *GPI*, and *MDH-2*) the probabilities that these individuals came from a population which was genetically common to *P. cygnus* were extremely low ($1.4\ 10^{-4}$, $4.9\ 10^{-3}$, and $2.2\ 10^{-6}$ respectively). Nei's (1978) unbiased measure of genetic identity separated *P. cygnus* from *P. longipes* at 0.759. The evidence supports genetic discreteness of the two species along the coast of Western Australia and thus supports the views of George (1962) that *P. cygnus* is a valid species.

Introduction

The Western Rock Lobster, *Panulirus cygnus*, inhabits the west coast of Australia approximately from Cape Leeuwin (34° 22'S) to North West Cape (21° 45'S). Seven other species of rock lobster also inhabit the west coast of Australia, but this zone is mainly occupied by *P. cygnus* with only minimal overlap with other species. *Jasus novaehollandiae* (Holthuis 1963, cited by Holthuis 1991) is sympatric with *P. cygnus* at the southern end of the range, while at the northern end there is an overlap with several tropical species, *P. versicolor* (Latreille 1804, cited by Holthuis 1991), *P. ornatus* (Fabricius 1798, cited by Holthuis 1991), and *P. penicillatus* (Olivier 1791, cited by Holthuis,1991). Occasional specimens of *P. longipes* have been recorded in this zone. The two other species are *P. polyphagus* (Herbst 1793, cited by Holthuis 1991) which occurs in the muddy water near Derby, and *P. homarus* (Linnaeus 1758, cited by Holthuis 1991) which inhabits turbid waters off the Pilbara coast (see Gray 1992).

Conjecture regarding the discreteness of *P. cygnus* has continued since 1912 (see Gray 1992). Some scientists believed that there were insufficient morphological differences (Glauert 1936; Sheard 1949; Chittleborough & Thomas 1969) as well as a lack of evidence of genetic isolation to warrant ranking *P. cygnus* as a distinct species. Rather, it was suggested that *P. cygnus* was part of a more widespread population of *P. longipes*, which is an Indo-west Pacific species (see Holthuis 1991). The subspecies *P. longipes longipes* is the western form, occurring from East Africa to Thailand, the Philippines and Indonesia. Occasional specimens of *P. l. longipes* are found along the coast of Western Australia within the zone of *P. cygnus*. The eastern subspecies *P.l. femoristriga* inhabits Japan, the Moluccas, New Guinea, Eastern Australia, New Caledonia, and Polynesia (Holthuis 1991).

Recognition of the western rock lobster as a distinct species has significant implications for the fishing industry. If it were part of the more widespread species, *P. longipes*, as suggested by Glauert (1936), Sheard (1949), and Chittleborough & Thomas (1969), then lobsters caught on the West Australian coast may have come from larvae drifting with ocean currents from elsewhere in the Indian and Pacific Oceans. Thus, protecting the breeding population on the coast of Western Australia may not be such a crucial management strategy. However, recognition of the western rock lobster as a discrete species *P. cygnus*, restricted to the waters off Western Australia, dictates that the fishery be managed accordingly.

Allozyme electrophoresis is a useful tool for distinguishing populations and species. One of the implications of a discrete population is that there is no gene-flow between it and other populations. Absence of gene-flow allows genetic differences to accumulate over generations through natural selection, random genetic drift, and mutation (Wallace 1981). This can result in speciation. Usually, distinct populations and/or species will show genetic differences (Altukov 1981; Johnson *et al.* 1993; Shaklee 1983). Intraspecific populations are distinguished in the most part by different frequencies of shared alleles, while species are distinguished by fixed allelic differences (Altukov 1981). Thompson *et al.* (1996) found no evidence of latitudinal genetic variation among populations of the western rock lobster from Garden Island to Shark Bay. The average F_{ST} among 5 sites over the 9 polymorphic loci studied was a very low 0.0002, which is consistent with current interpretations that the western rock lobster is a single panmictic population (see Thompson *et al.* 1996). The aim of this study was to investigate the genetic discreteness of *P. cygnus* and *P. longipes* along the coast of Western Australia.

Table 1

Enzymes and electrophoretic buffers used to study genetic variation in *P. cygnus* and *P. longipes*. Buffer recipes are given in Hillis & Moritz (1990).

Enzyme	E.C. number	Locus abbreviation	Buffer
Arginine phosphokinase	2.7.3.3	*APK*	TC8
Esterase	3.1.1.–	*EST*	TC8
Glucose phosphate isomerase	5.3.1.9	*GPI*	LiOH
Leucylproline peptidase	3.4.–.–	*LPP*	TC8
Leucylglycylglycine peptidase	3.4.1.3	*LGG*	TC8
Malate dehydrogenase	1.1.1.37	*MDH-1, MDH-2*	TM
Mannosephosphate isomerase	5.3.1.8	*MPI*	TC8
6-Phosphogluconate dehydrogenase	1.1.1.44	*6PGD*	TM
Phosphoglucomutase	2.7.5.1	*PGM*	TM
Valylleucine peptidase	3.4.–.–	*VLP*	TC8

Materials and Methods

A leg from each of 455 adult rock lobsters (*P. cygnus*) was collected during 1994. Rock lobsters were collected at commercial depots at 5 locations; Garden Island (n=84), Two Rocks (n=81), Geraldton (n=97), Southern Abrolhos (n=99) and Shark Bay (n=94). These samples were frozen immediately in liquid nitrogen before being transferred to a freezer at -70°C. Tissue samples (muscle) from three *P. longipes longipes* were collected during 1995, also from a commercial depot. The *P. longipes* specimens were characterised by the presence of pale spots on the legs. These specimens were caught off Shark Bay, within the zone of *P. cygnus*. They were frozen immediately in liquid nitrogen and later stored at -70°C.

Ten enzyme systems representing 11 loci were scored in both *P. cygnus* and *P. longipes* (Table 1). Electrophoretic procedures are described in more detail by Thompson *et al.* (1996).

Figure 1. Isozyme formation of PGM showing the fixed allelic differences of the *P. longipes* specimens (positions 2–4) compared to *P. cygnus* (positions 1, 5–8). Individual genotypes from left to right are: 22, 34, 34, 33, 22, 22, 22, 22.

Expected genotypic frequencies at each locus were calculated using BIOSYS-1.7 (Swofford & Selander 1981). The genotypic frequencies at each locus from the pooled *P. cygnus* population (since Thompson *et al.* 1996 found no evidence of genetic subdivision) were used as probability estimates for the observed genotypic frequencies of the *P. longipes* individuals. The expected probability of a *P. longipes* individual having a particular genotype was equal to the expected frequency of that genotype occurring in the *P. cygnus* population.

The genetic differentiation among populations was examined using BIOSYS-1.7 (Swofford & Selander 1981). Clustering was performed based on the methods of Nei's (1978) unbiased genetic identity.

Results

One locus, *PGM*, showed unique alleles and genotypes between the 2 groups (Figure 1, Table 2). At ten loci, the 3 *P. longipes* specimens had alleles common

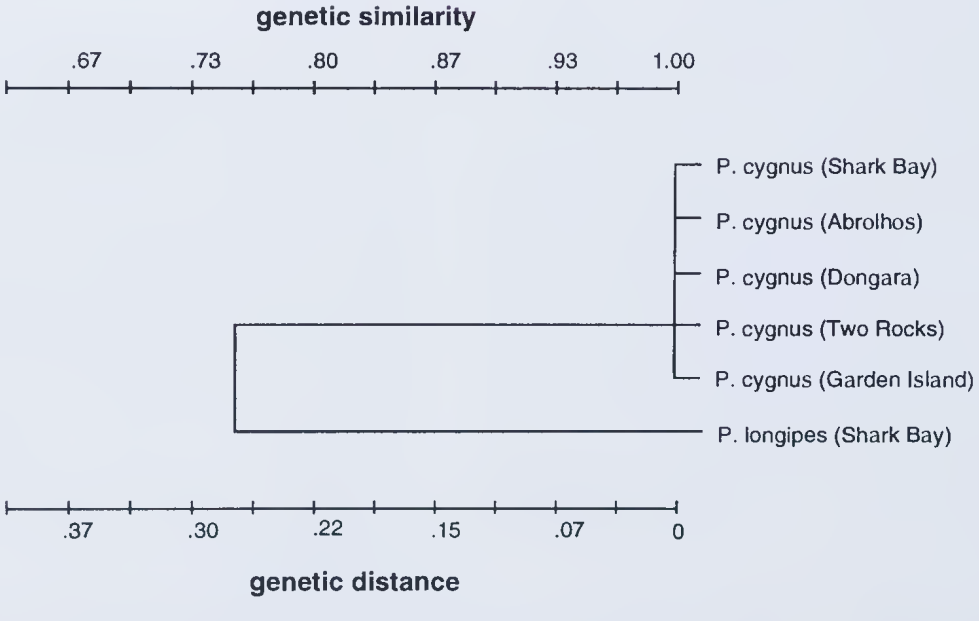

Figure 2. Dendrogram based on Nei's (1978) unbiased genetic identities of *P. cygnus* and *P. longipes* caught off the coast of Western Australia.

Table 2

Genotypic classes of *P. cygnus* with their associated observed frequencies, and the individual genotypes scored for each *P. longipes* specimen with the associated probabilities that these animals came from a population that was genetically common to *P. cygnus*. N is sample size.

P. cygnus (N = 455)			*P. longipes* (N = 3)		
Locus	Genotypes	Frequency	Locus	Genotypes	Probability
APK	2–2	1	*APK*	2–2, 2–2, 2–2	1
EST	1–1	0.024	*EST*	1–1, 1–1, 1–1	$1.4\ 10^{-4}$
	1–2	0.229			
	1–3	0.747			
GPI	1–1	$2.198\ 10^{-3}$	*GPI*	2–2, 2–2, 2–2	$4.9\ 10^{-3}$
	1–2	$4.396\ 10^{-3}$			
	1–3	$8.791\ 10^{-3}$			
	2–2	0.1692			
	2–3	0.4132			
	3–3	0.4022			
IDH	2–2	1	*IDH*	2–2, 2–2, 2–2	1
LGG	2–2	1	*LGG*	2–2, 2–2, 2–2	1
LPP	1–2	0.0202	*LPP*	2–2, 2–2, 2–2	0.9415
	2–2	0.9801			
MDH-1	1–2	$6.593\ 10^{-3}$	*MDH-1*	2–2, 2–2, 2–2	0.9609
	2–2	0.9868			
	2–3	$6.593\ 10^{-3}$			
MDH-2	1–1	$1.32\ 10^{-2}$	*MDH-2*	1–1, 1–1, 1–1	$2.2\ 10^{-6}$
	1–2	0.1165			
	2–2	0.8615			
	2–3	$8.80\ 10^{-3}$			
MPI	2–2	0.9956	*MPI*	2–2, 2–2, 2–2	0.9869
	2–3	$4.3956\ 10^{-3}$			
PGM	1–2	$1.538\ 10^{-2}$	*PGM*	3–3, 3–4, 3–4	0
	2–2	0.9846			
VLP	1–2	1.32×10^{-2}	*VLP*	2–2, 2–2, 2–2	0.8683
	2–2	0.9540			
	2–3	$3.330\ 10^{-3}$			

to both species. However, the probabilities of them coming from a population which was genetically linked to *P. cygnus* were extremely small at the *EST*, *GPI*, and *MDH-2* loci (Table 3). At the other 7 loci (*APK*, *IDH*, *LGG*, *LPP*, *MDH-1*, *MPI*, and *VLP*) the 3 *P. longipes* specimens shared alleles, with no evidence of differences between the 2 groups. Nei's (1978) genetic identity cluster analysis clearly separted the *P.longipes* specimens from all *P. cygnus* populations at 0.759 (Figure 2).

Discussion

Even though only 3 individual *P. longipes* were used in this study, it is clear that these animals came from a population that is not genetically linked (*i.e.* there is no gene-flow) to *P. cygnus*. *PGM* was the definitive locus for this conclusion with completely unique alleles and genotypes observed in all 3 *P. longipes* specimens. Even if there was only a small amount of gene-flow between the 2 groups, one would expect rare genotypes (or at least alleles) to occur in 455 animals. This was not the case. Furthermore, Nei's (1978) unbiased estimate of genetic identity separated the 2 species at 0.759. In a study of the freshwater crayfish genus *Cherax*, 6 species were separated with genetic identities ranging from 0.92 to 0.53 (Austin & Knott 1996). The electrophoretic results here provide the evidence of genetic isolation between *P. cygnus* and *P. longipes* along the coast of Western Australia that was lacking at the time of Chittleborough & Thomas (1969).

Phillips (1981) reported that larvae of *P. cygnus* are transported offshore through a combination of behavioural adaptations resulting in the early phyllosoma being at the surface at nights, and surface currents (summer pattern of offshore wind drift) during the early stage of their life-cycle. Mid to late stages are entrained within mesoscale eddies and gyres up to 1000 km offshore. Final stages are returned by a change in behaviour (spending more time at greater depth) in conjunction with the deeper onshore flow. Pollock (1992) presumed from oceanographic characteristics that the larval pool of *P. cygnus* off the coast of Western Australia resulted in separation and ultimately in speciation of this group. The genetic evidence supports the notion that *P. cygnus* is genetically isolated from *P. longipes* along the coast of Western Australia.

However, larval transport processes occasionally result in larvae being moved long distances. The *P. longipes* collected for this study presumably came to the coast of Western Australia via larval transport rather than by juvenile migration. This shows the possibility for gene-flow between *P. cygnus* and *P. longipes*. Phillips *et al.* (1980) proposed that near-adult specimens of *P. cygnus* with pale spots on their legs (characteristic of specimens of *P. longipes* from type locality of Zanzibar) were in fact hybrids due to gene-flow from the north. More likely, these individuals were foreign larvae which managed to metamorphose to become juveniles.

The foreign *P. longipes* specimens negate the hypothesis of Pollock (1992) that larvae from each species only metamorphose into the settling puerulus stage when they experience physical, and/or chemical cues native to endemic environments. It is more probable that species are separated due to physical isolation (*i.e.* entrainment of larval pools) rather than due to the failure of foreign larvae to metamorphose. Larvae of *P. longipes* may occasionally be transported and even settle in the waters off Western Australia, but the genetic evidence supports a lack of significant transfer of genes between these groups.

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